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A MEANS FOR THE PROPHYLACTIC AND THERAPEUTIC TREATMENT
OF GROUP A STREPTOCOCCAL INFECTIONS

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DESCRIPTION

5 BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to an oral delivery means, such as a candy, chewing gum, lozenge, troche, tablet, a powder, an aerosol, a liquid or a liquid spray, containing a group C streptococcal phage associated lysin enzyme for the prophylactic and therapeutic treatment of Streptococcal A throat infections, commonly known as strep throat.

2. Description of the Prior Art

Group A streptococci have been shown to be an important pathogen capable of existing both in a carrier state in an asymptomatic individual and in a symptomatic individual with symptoms of disease ranging from a mild sore throat, tonsillitis, or impetigo. If untreated these streptococcal infections could lead to glomerulonephritis, rheumatic fever and possibly permanent rheumatic heart disease. With the advent of antimicrobial agents, specifically penicillin derived antibiotics, the causative organism can be readily eliminated following the prescribed regimen of appropriate antibiotic therapy.

The fact that an infected individual (usually children & young adults) can pass group A streptococcal organisms to others,

particularly in daycare centers and schools, necessitates the isolation of the known infected individual away from these environments for at least 24 to 72 hours after antimicrobial therapy has been initiated. It has been shown in controlled studies that early detection and appropriate treatment results in a reduction in the overall pattern of cyclic transmission of the troublesome pathogen as well as a reduction or elimination of the sequelae of group A infections (rheumatic fever or nephritis).

The first individual to identify the serological and immunological groups of streptococci was Dr. Rebecca Lancefield, (Lancefield, R. C., "A Serological Differentiation of Human and other Groups of Hemolytic Streptococci," J. Exp. Med., Vol. 57, pp 571-595 1933), after whom the grouping system was named. The group A streptococcus was identified on the basis of B-1, 4 N-acetylglucosamine terminal sugar moieties on a repeating rhamnose sugar backbone found as part of the structure of the organism's cell wall. Antiserum raised against group A streptococci and subsequent absorptions to remove cross-reactions were shown to specifically react with the cell wall component of these organisms and became the grouping antisera for group A streptococci. A number of methods have been devised to fragment the group A streptococcal cell wall carbohydrate. These methods include heating by boiling at pH 2.0, autoclaving, trichloroacetic acid extraction, hot formamide digestion, nitrous acid extraction and enzyme digestion by enzymes derived from the soil microorganisms of species streptomyces, and the phage-associated

enzyme lysin. Each of these methods have various advantages and disadvantages.

5 The rapid diagnosis of group A streptococcal pharyngitis has become more readily available to both physicians and clinical laboratories by replacing time consuming culturing methods requiring a minimum of 24 to 72 hours to identify the presence of group A streptococci with a rapid antigen-antibody test capable of being performed and read in less than one hour. Culturing methods vary in the degree of sensitivity of detection. In one case, a simple 5% sheep blood agar plate may be used in conjunction with a Bacitracin disc and culturing 24 hours at 37 degree(s) C. aerobically to identify group A streptococci. Alternatively, a selective media and anaerobic conditions may be used to inhibit overgrowth by other organisms and incubation at 35 degree(s) C. for a minimum of 48 hours. In addition, depending on the transport media, the delay in testing, and any antibacterial agents that the patient may have taken, culturing may result in nonviable organisms that fail to grow in the media although the patient is indeed colonized by the group A streptococcus. In the latter case a sensitive immunoassay for group A streptococcal antigen can detect these nonviable organisms.

25 The sensitivity of the immunoassay procedure is effected by the amount of group A streptococcal carbohydrate antigen released and recognized by the specific immunological reagent. Enzymatic digestion by enzymes such as that produced by the species

streptomyces, have proven to be quite effective in antigen release over some chemical methods and micro-nitrous acid but the poor specific activity and the presence of proteases makes it slow and incompatible for prolonged contact with immunological reagents.

5 Maxted, (Maxted, W. R., "The Active Agent in Nascent Phage Lysis of Streptococci," J. Gen Micro, vol 16, pp 585-595 1957), Krause, (Krause, R. M., "Studies on the Bacteriophages of Hemolytic Streptococci," J. Exp Med, vol 108, pp 803-821 1958), and Fischetti, (Fischetti, V. A., et al, "Purification and
10 Physical Properties of Group C Streptococcal Phage Associated Lysin," J. Exp Med, Vol 133 pp 1105-1117 1971, have reported the characteristics of an enzyme produced by the group C streptococcal organism after being infected with a particular bacteriophage identified as C1. The enzyme was given the name
15 lysin and was found to specifically cleave the cell wall of group A, group C and group E streptococci. These investigators provided information on the characteristics and activities of this enzyme with regards to lysing the group A streptococci and releasing the cell wall carbohydrate. They never reported on
20 the utility of this enzyme in an immunological diagnostic test for the detection of group A streptococci from throat swabs in patients. The failure to use this enzyme for a clinical diagnostic test was due to a number of problems associated with the enzyme such as: the difficulty in growing large amounts of
25 bacteriophage in the group C streptococci, the time delays in inactivating the residual enzyme when trying to obtain phage

stocks, the instability of the enzyme itself to oxidative conditions and heat, and nonspecific reactions in immunoassays performed in the presence of other organisms and the biological components in the sample.

5 U.S. Patent No. 5,604,109 (Fischetti et al.) teaches the rapid and sensitive detection of group A streptococcal antigens by a diagnostic test kit which utilizes a sampling device consisting of a throat swab made of synthetic or natural fibers such as Dacron or rayon and some type of shaft which holds the fibers and
10 which is long enough to place the fibers in the tonsillar area and capable of being used to swab the area to remove sufficient numbers of colonizing or infecting organisms. The swab can then be placed in the enzyme extraction reagent in several configurations and subsequently used in an immunoassay. The
15 invention can comprise a test kit for detecting Group A streptococci, comprising an extraction reagent containing lysin enzyme for releasing Group A streptococcal components, and a ligand capable of binding with a component of the Group A streptococcus.

20 SUMMARY OF THE INVENTION

The present invention (which incorporates U.S. Patent No. 5,604,109 in its entirety by reference) uses the lysin enzyme produced by the group C streptococcal organism after being infected
25 with a particular bacteriophage (identified as C1) as either a prophylactic treatment for preventing those who have been exposed

to others who have the symptoms of a strep infection from getting sick, or as a therapeutic treatment for those who have already become ill from the infection.

The lysin enzyme would be administered in the form of a candy, chewing gum, lozenge, troche, tablet, a powder, an aerosol, a liquid or a liquid spray.

The method for the treatment of streptococcus A exposure will comprise applying an effective dosage of a pharmaceutically acceptable amount of an extraction reagent comprising Group C streptococcal phage associated lysin enzyme to the oral mucosa of a mammal in need of treatment, permitting the extraction reagent to remain in contact with the oral mucosa for a period of time necessary for the lysin enzyme to saturate the oral mucosa; and applying additional dosages of such the lysin enzyme in like fashion until treatment is complete.

The present invention is based upon the discovery that phage lysin can effectively and efficiently break down the cell wall of Group A Streptococci and the resultant antigenic fragments are reactive with antibodies specific for the Group A Streptococcal carbohydrate. The semipurified enzyme is lacking in proteolytic enzymatic activity and therefore non-destructive to specific antibodies when present during the digestion of the bacterial cell wall.

DETAILED DESCRIPTION OF THE INVENTION

This invention discloses the use of the lysin enzyme produced by the group C streptococcal organism after being infected with a particular bacteriophage (identified as C1) as either a prophylactic treatment for preventing those who have been exposed to others who have the symptoms of a strep infection from getting sick, or as a therapeutic treatment for those who have already become ill from the infection.

Means of application include, but are not limited to direct, indirect, carrier and special means or any combination of means. Direction application of the lysin enzyme may be by nasal sprays, nasal drops, nasal ointments, nasal washes, nasal injections, nasal packings, or indirectly through use of throat lozenges, or through use of mouthwashes or gargles, or through the use of ointments applied to the nasal nares, the bridge of the nose, or the face or any combination of these and similar methods of application. The forms in which the lysin enzyme may be administered include but are not limited to lozenges, troches, candies, injectants, chewing gums, tablets, powders, sprays, liquids, ointments, and aerosols.

Prior to, or at the time the enzyme is put in a carrier system, the enzyme may be in a phosphate buffer environment for maintaining a pH range between about 4.0 and about 8.0, more preferably between about 5.5 and about 7.5 and more preferably at about 6.1.

The stabilizing buffer should allow for the optimum activity of the lysin enzyme. The buffer may be a reducing reagent, such as dithiothreitol. The stabilizing agent may also be or include a

metal chelating reagent, such as ethylenediaminetetraacetic acid disodium salt, or may also contain a citrate-phosphate buffer.

To prevent spoilage, the stabilizing buffer may also contain a bactericidal or bacteriostatic reagent as a preservative, such as a small amount of sodium benzoate.

The lozenge into which the lysin enzyme is added may contain any or all of the following ingredients: sugar, corn syrup, a variety of dyes, non-sugar sweeteners, flavorings, and any binders. Similarly, any gum based products may contain any or all of the following ingredients: acacia, carnauba wax, citric acid, corn starch, food colorings, flavorings, non-sugar sweeteners, gelatin, glucose, glycerin, gum base, shellac, sodium saccharin, sugar, water, white wax, and cellulose and other binders.

Lozenges may contain any or all of the following ingredients: sucrose, corn starch, acacia, gum tragacanth, anethole, linseed, oleoresin, mineral oil, and cellulose and other binders. In another embodiment of the invention, sugar substitutes are used in place of dextrose, sucrose, or other sugars.

Any of the carriers for the lysin enzyme may be manufactured by conventional means. However, it is preferred that any mouthwash or similar type products not contain alcohol to prevent denaturing of the enzyme. Similarly, when the lysin enzyme is being placed in a cough drop, gum, candy or lozenge during manufacture, such placement should be made prior to the hardening of the lozenge or candy but after the cough drop or candy has cooled somewhat, to avoid heat denaturation of the enzyme.

The enzyme may be added to these substances in liquid form or in a lyophilized state, whereupon it will be solubilized when it meets a liquid body. The enzyme may also be in a micelle or liposome.

5 The effective dosage rates of the use of the lysin enzyme will depend in part on whether the lysin will be used therapeutically or prophylactically, the duration of exposure of the recipient to the Streptococci, the size and weight of the patient, etc. The effective dosage comprises an amount which is sufficient to provide
10 an enzyme concentration of between about .1 mM and 1 M in the saliva of the mammal being treated, or between about 1 mM and about 300 mM in the saliva of the mammal being treated, or between about 5 mM and about 50 mM in the saliva of the mammal being treated. While this treatment may be used in any mammalian species, the
15 preferred use of this product is for a human.

EXAMPLE 1

20 The extraction reagent containing the group C phage lysin enzyme is prepared as follows:

25 Group C streptococcal strain 26RP66 (ATCC #21597) or any other group C streptococcal strain is grown in Todd Hewitt medium at 37 degree(s) C. to an OD of 0.23 at 650 nm in an 18 mm tube. Group C bacteriophage (C1) (ATCC #21597-B1) at a titer of 5X10 sup 6 is added at a ratio of 1 part phage to 4 parts cells. The mixture is allowed to remain at 37 degree(s) C. for 18

min at which time the infected cells are poured over ice cubes to reduce the temperature of the solution to below 15 degree(s) C. The infected cells are then harvested in a refrigerated centrifuge and suspended in 1/300th of the original volume in 0.1M phosphate buffer, pH 6.1 containing 5×10^{-3} M dithiotreitol and 10 μ g of DNAase. The cells will lyse releasing phage and the lysin enzyme. After centrifugation at 100,000X g for 5 hrs to remove most of the cell debris and phage, the enzyme solution is aliquoted and tested for its ability to lyse Group A Streptococci.

The number of units/ml in a lot of enzyme is determined to be the reciprocal of the highest dilution of enzyme required to reduce the OD₆₅₀ of a suspension of group A streptococci at an OD of 0.3 to 0.15 in 15 minutes. In a typical preparation of enzyme 4×10^5 to 4×10^6 units are produced in a single 12 liter batch.

Use of the enzyme requires a minimum number of units of lysin enzyme per test depending on the incubation times required. The enzyme is diluted in a stabilizing buffer containing the appropriate conditions for stability, maximum enzymatic activity. The preferred embodiment is to use a lyophilized reagent which can be reconstituted with water. The stabilizing buffer can comprise a reducing reagent, which can be dithiothreitol in a concentration from 0.001M to 1.0M, preferably 0.005M. The stabilizing buffer can comprise a metal chelating reagent, which can be ethylenediaminetetraacetic acid disodium salt in a concentration from 0.00001M to 1.0M,

preferably 0.005M. The stabilizing buffer can comprise a citrate-phosphate buffer in a concentration from 0.001M to 1.0M, preferably 0.05M. The stabilizing buffer can have a pH value in the range of from about 4.0 to 8.0, preferably 6.1. The stabilizing buffer can comprise a bactericidal or bacteriostatic reagent as a preservative. Such preservative can be sodium azide in a concentration from 0.001 percent to 0.1 percent, preferably 0.02 percent.

The preparation of phage stocks for lysin production is the same procedure described above for the infection of phage and group C streptococcus in the preparation of the lysin enzyme. However, instead of pouring the infected cells over ice, the incubation at 37 degree(s) C. is continued for a total of 1 hour to allow lysis and release of the phage and also enzyme in the total volume. In order for the phage to be used for subsequent lysin production the residual enzyme must be inactivated or removed to prevent lysis from without of the group C cells rather than phage infection.

EXAMPLE 2

The enzyme prepared according to example 1 is diluted to a concentration of 100 units/ml in a buffer consisting of 0.05M citrate phosphate buffer pH 6.1 containing 0.1% rabbit immunoglobulin, 0.005M (ethylenedinitrilo) tetraacetic acid disodium salt (EDTA), 0.005M Dithiothreitol, 0.02% sodium azide, 0.01% N-acetylglucosamine. One part colloidal gold sol labelled with Group A Streptococcal Antibody (OD sup 520 1.5) suspended

in 0.02M Tris buffer pH 8.2, 1.0% bovine serum albumin, 0.02% sodium azide, 300K units heparin, is added to 3 parts of the enzyme reagent, mixed, filtered through a 0.22 micron filter, and 200 microliters aliquoted per tube and lyophilized. This lyophilized reagent is stable at elevated temperatures (i.e. 45 degree(s) C.) for short term conditions (i.e. 2 weeks) and long term storage at room temperatures (>1 year).

EXAMPLE 3

Method

1. Start a day culture of group A streptococcal strain S43/192/39R (Streptomycin resistant) (from frozen blood broth); 500 μ l in 50 ml of Todd Hewitt (TH) broth containing 1 % yeast extract and 100 μ l of Streptomycin/ml.
2. Grow to an OD₆₅₀ of 0.59
3. Centrifuge for 15 minutes at 3000 rpm to sediment bacteria.
4. Resuspend organisms in 1 ml volume of TH w/o antibiotics (3 x 10⁵/100 μ l as determined by plate count).
5. Add 0.5 of these concentrated cells to 0.5 ml of pH 6.1 phosphate buffer as a control.
6. Five minutes before administering to the mice, 0.5 ml of the concentrated cell suspension was mixed with 0.5 ml of phage lysin solution pre-diluted to 10,000 units/ml in pH 6.1 phosphate buffer.

Five mice received 60 μ l of "control" solution divided equally orally and intranasally.

Five mice received 60 μ l of lysin and bacteria mixture divided equally orally and intranasally.

Throat swabs were performed onto 5% sheep blood, proteose peptone agar plates containing 500 μ g/ml of streptomycin. Plates were incubated overnight at 37° C.

The following results were obtained:

	7/22 1d	7/23 2d	7/24 3d	7/28 7d
	Colony Forming Units			
LYSIN				
L1	0	0	0	0
L2	0	0	0	0
L3	0	0	0	0
L4	0	1	0	0
L5	0	0	1	0
CONTROL				
C1	26	14	7	0
C2	>400	17	100	83
C3	9	0	15	0
C4	>400	>400	>400	220
C5	2	2	30	0

These results show that the contact between phage lysin and group A streptococci for as little as five minutes prevents the streptococci from colonizing the upper respiratory tract of the mice in this model system.

Each dose is kept in contact with the oral mucosa as long as necessary in order to improve performance over the original invention. Improved administration of the lysin enzyme to the oral

mucosa may be by any means such as gargles, mouth rinses, lozenges, troches, chewing gums, candies, powders, and sprays, so long as it offers convenience, palatability, safety, or further reduction in the duration of or prevention of the effects of Streptococcus A exposure.

Many modifications and variations of the present invention are possible in light of the above teachings. It is, therefore, to be understood within the scope of the appended claims the invention may be protected otherwise than as specifically described.

What is claimed is:

1. A treatment for streptococcal throat infection comprising:

An extraction reagent comprising Group C streptococcal
phage associated lysin enzyme; and

an oral delivery mode for delivering said Group C
streptococcal phage associated lysin enzyme, said group C
streptococcal phage associate lysin enzyme being in said oral
delivery mode.

2. The treatment for streptococcal throat infection
according to claim 1, wherein said oral delivery mode is selected
from the group consisting of a candy, chewing gum, lozenge, troche,
tablet, a powder, an aerosol, a liquid and a liquid spray.

3. The treatment for streptococcal throat infection
according to claim 1, further comprising:

a phosphate buffer for maintaining the enzyme in an
environment having pH range between about 4.0 and about 8.0.

4. The treatment for streptococcal throat infection
according to claim 3, further comprising:

the phosphate buffer for maintaining the enzyme in an
environment having a PH range between about 5.5 and 7.5.

5. The treatment for streptococcal throat infection according to claim 2, further comprising a sugar substitute in said oral delivery mode.

5 6. A method for the treatment of streptococcus A exposure comprising:

applying an effective dosage of a pharmaceutically acceptable amount of:

10 an extraction reagent comprising Group C streptococcal phage associated lysin enzyme to the oral mucosa of a mammal in need of treatment;

15 permitting the extraction reagent to remain in contact with the oral mucosa for a period of time necessary for the lysin enzyme to saturate the oral mucosa; and

20 applying additional dosages of such the lysin enzyme in like fashion until treatment is complete.

7. The method for the treatment of streptococcus A exposure according to claim 6, wherein said treatment is therapeutic.

8. The method for the treatment of streptococcus A exposure according to claim 6, wherein said treatment is prophylactic.

25 9. The method for the treatment of streptococcus A exposure according to claim 6 wherein an effective dosage comprises an amount which is sufficient to provide an enzyme concentration of

between about .1 mM and 1 M in the saliva of the mammal being treated.

10. The method for the treatment of streptococcus A exposure according to claim 9, wherein the effective dosage comprises the amount which is sufficient to provide the enzyme concentration of between about 1 mM and about 300 mM in the saliva of the mammal being treated.

11. The method for the treatment of streptococcus A exposure according to claim 10, wherein the effective dosage comprises the amount which is sufficient to provide the enzyme concentration of between 5 mM and about 50 mM in the saliva of the mammal being treated.

12. The method for the treatment of streptococcus A exposure according to claim 6, wherein the mammal being treated is a human.

13. The method for the treatment of streptococcus A exposure, according to claim 6, wherein a dosage form is selected from the group consisting of a candy, chewing gum, lozenge, troche, tablet, a powder, an aerosol, a liquid and a liquid spray.

14. The method for the treatment of streptococcus A exposure according to claim 6, wherein said extraction free agent comprises

a stabilizing buffer to allow optimum activity of said lysin enzyme.

15. The method of claim 14, wherein the stabilizing
5 buffer comprises a reducing reagent.

16. The method of claim 15, wherein the reducing reagent is dithiothreitol.

10 17. The method of claim 14, wherein said extraction reagent is a lyophilized reagent which is reconstituted with liquid.

15 18. The method of claim 14, wherein the stabilizing buffer comprises a metal chelating reagent.

19. The method of claim 14, wherein the metal chelating reagent is ethylenediaminetetraacetic acid disodium salt.

20 20. The method of claim 14, wherein the stabilizing buffer comprises a citrate-phosphate buffer.

21. The method of claim 14, wherein the stabilizing buffer has a pH value in the range from 5.0 to 9.0.

22. The method of claim 14, wherein the stabilizing buffer comprises a bactericidal or bacteriostatic reagent as a preservative.

5 23. An oral delivery means for the therapeutic or prophylactic treatment of Streptococcal C exposure, said means comprising:
Group C streptococcal phage associated lysin enzyme; and
an oral delivery mode for delivering said Group C streptococcal phage associated lysin enzyme, said Group C
10 streptococcal phage associate lysin enzyme being in said oral delivery mode.

15 24. The oral delivery means of claim 23 wherein an effective dosage comprises an amount which is sufficient to provide an enzyme concentration of between about .1 mM and 1 M in the saliva of the mammal being treated.

20 25. The oral delivery means of claim 23, wherein said oral delivery mode is selected from the group consisting of a candy, chewing gum, lozenge, troche, tablet, a powder, an aerosol, a liquid and a liquid spray.

1990-1991		1991-1992		1992-1993		1993-1994		1994-1995		1995-1996		1996-1997		1997-1998		1998-1999		1999-2000		2000-2001		2001-2002		2002-2003		2003-2004		2004-2005		2005-2006		2006-2007		2007-2008		2008-2009		2009-2010		2010-2011		2011-2012		2012-2013		2013-2014		2014-2015		2015-2016		2016-2017		2017-2018		2018-2019		2019-2020		2020-2021		2021-2022		2022-2023		2023-2024		2024-2025		2025-2026		2026-2027		2027-2028		2028-2029		2029-2030		2030-2031		2031-2032		2032-2033		2033-2034		2034-2035		2035-2036		2036-2037		2037-2038		2038-2039		2039-2040		2040-2041		2041-2042		2042-2043		2043-2044		2044-2045		2045-2046		2046-2047		2047-2048		2048-2049		2049-2050		2050-2051		2051-2052		2052-2053		2053-2054		2054-2055		2055-2056		2056-2057		2057-2058		2058-2059		2059-2060		2060-2061		2061-2062		2062-2063		2063-2064		2064-2065		2065-2066		2066-2067		2067-2068		2068-2069		2069-2070		2070-2071		2071-2072		2072-2073		2073-2074		2074-2075		2075-2076		2076-2077		2077-2078		2078-2079		2079-2080		2080-2081		2081-2082		2082-2083		2083-2084		2084-2085		2085-2086		2086-2087		2087-2088		2088-2089		2089-2090		2090-2091		2091-2092		2092-2093		2093-2094		2094-2095		2095-2096		2096-2097		2097-2098		2098-2099		2099-2100		2100-2101		2101-2102		2102-2103		2103-2104		2104-2105		2105-2106		2106-2107		2107-2108		2108-2109		2109-2110		2110-2111		2111-2112		2112-2113		2113-2114		2114-2115		2115-2116		2116-2117		2117-2118		2118-2119		2119-2120		2120-2121		2121-2122		2122-2123		2123-2124		2124-2125		2125-2126		2126-2127		2127-2128		2128-2129		2129-2130		2130-2131		2131-2132		2132-2133		2133-2134		2134-2135		2135-2136		2136-2137		2137-2138		2138-2139		2139-2140		2140-2141		2141-2142		2142-2143		2143-2144		2144-2145		2145-2146		2146-2147		2147-2148		2148-2149		2149-2150		2150-2151		2151-2152		2152-2153		2153-2154		2154-2155		2155-2156		2156-2157		2157-2158		2158-2159		2159-2160		2160-2161		2161-2162		2162-2163		2163-2164		2164-2165		2165-2166		2166-2167		2167-2168		2168-2169		2169-2170		2170-2171		2171-2172		2172-2173		2173-2174		2174-2175		2175-2176		2176-2177		2177-2178		2178-2179		2179-2180		2180-2181		2181-2182		2182-2183		2183-2184		2184-2185		2185-2186		2186-2187		2187-2188		2188-2189		2189-2190		2190-2191		2191-2192		2192-2193		2193-2194		2194-2195		2195-2196		2196-2197		2197-2198		2198-2199		2199-2200		2200-2201		2201-2202		2202-2203		2203-2204		2204-2205		2205-2206		2206-2207		2207-2208		2208-2209		2209-2210		2210-2211		2211-2212		2212-2213		2213-2214		2214-2215		2215-2216		2216-2217	
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Applicant or Patentee: New Horizons Diagnostics, Inc. Inventors - Lawrence Loomis and Vincent Fischetti

Serial or Patent No.: _____ Atty. Dkt. No. NewH-1

Filed or Issued: _____

A MEANS FOR THE PROPHYLACTIC AND THERAPEUTIC TREATMENT OF GROUP A STREPTOCOCCAL INFECTION

**VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS
(37 CFR 1.9(f) AND 1.27 (c)) — INDEPENDENT INVENTOR**

As a below named inventor, We hereby declare that We qualify as an independent inventor as defined in 37 CFR 1.9(c) for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, to the Patent and Trademark Office with regard to the invention entitled A Means for the Prophylactic and Therapeutic Treatment of Group A Streptococcal Infection, described in

(☒) the specification filed herewith

() application serial no. _____, filed _____

() patent no. _____, issued _____

I have not assigned, granted, conveyed or licensed and am under no obligation under contract or law to assign, grant, convey, or license any rights in the invention to any person who could not be classified as an independent inventor under 37 CFR 1.9(c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below.

() no such person, concern or organization

(X) persons, concerns or organizations listed below*

* NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

FULL NAME New Horizons Diagnostics, Inc.

ADDRESS 9110 Red Branch Road, Columbia, Maryland 21045-2014

() INDIVIDUAL (X) SMALL BUSINESS CONCERN () NONPROFIT CORPORATION

FULL NAME _____

ADDRESS _____

() INDIVIDUAL () SMALL BUSINESS CONCERN () NONPROFIT CORPORATION

FULL NAME _____

ADDRESS _____

() INDIVIDUAL () SMALL BUSINESS CONCERN () NONPROFIT CORPORATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate: (37 CFR 1.28(b)).

* I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Vincent Fischetti, Ph.D.

Lawrence Loomis

NAME OF INVENTOR

NAME OF INVENTOR

NAME OF INVENTOR

Signature of Inventor

Signature of Inventor

Signature of Inventor

DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

As a below named inventor, we hereby declare that:

Our residence, post office address and citizenship are as stated below next to my name.

We believe that we are the original, first and joint inventors of the subject matter which is claimed and for which a patent is sought on the invention entitled A LOZENGE FOR THE PROPHYLACTIC AND THERAPEUTIC TREATMENT OF GROUP A STREPTOCOCCAL INFECTIONS, the specification of which:

(check one) ☒ is attached hereto ☐ being filed on _____ as
Application Serial No. _____ and
was amended on _____
(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)

Priority Claimed

(NUMBER)

(COUNTRY)

(DAY/MONTH/YEAR FILED) ☐ YES ☐ NO

(NUMBER)

(COUNTRY)

(DAY/MONTH/YEAR FILED) ☐ YES ☐ NO

(NUMBER)

(COUNTRY)

(DAY/MONTH/YEAR FILED) ☐ YES ☐ NO

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §156(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this applications:

(APPLICATION SERIAL NO.)

(FILING DATE)

(STATUS)
(PATENTED, PENDING, ABANDONED)

(APPLICATION SERIAL NO.)

(FILING DATE)

(STATUS)
(PATENTED, PENDING, ABANDONED)

POWER OF ATTORNEY: As a named inventors, We hereby appoint the following attorneys and/or agents to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

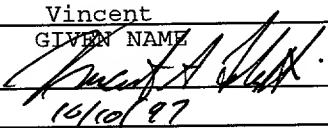
SEND CORRESPONDENCE TO:

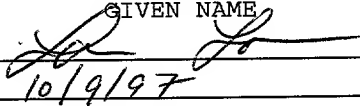
Jonathan E. Grant
Grant Patent Services
2213 M Street, N.W.
Suite 300
Washington, D.C. 20037

DIRECT TELEPHONE CALLS TO:

Jonathan E. Grant
(301) 608-8078

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine and imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or document or any patent issuing thereon.

Full name of sole or first inventor Vincent Fischetti
GIVEN NAME MIDDLE NAME FAMILY NAME
 Inventor's signature 
 Date of signature 10/10/97
 Residence West Hempstead New York U.S.A.
CITY STATE OR PROVINCE COUNTRY
 Citizenship U.S.A.
 Post Office Address 448 Joan Court
 (insert complete mailing address, including country) West Hempstead, New York 11552

Full name of second inventor Lawrence Loomis
GIVEN NAME MIDDLE NAME FAMILY NAME
 Inventor's signature 
 Date of signature 10/9/97
 Residence Columbia Maryland U.S.A.
CITY STATE OR PROVINCE COUNTRY
 Citizenship U.S.A.
 Post Office Address 113374 Buckelberry Path
 (insert complete mailing address, including country) Columbia, Maryland 21044 U.S.A.

Applicant or Patentee: New Horizons Diagnostics, Inc., Inventors - Lawrence Loomis and Vincent Fischetti, Ph.D.

Serial or Patent No.: _____ Atty. Dkt. No. NewH-2

Filed or Issued: _____

For: A MEANS FOR THE PROPHYLACTIC AND THERAPEUTIC TREATMENT OF GROUP A STREPTOCOCCAL INFECTION

**VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS
(37 CFR 1.9(f) AND 1.27 (c)) — SMALL BUSINESS CONCERN**

I hereby declare that I am

- () the owner of the small business concern identified below:
(X) an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN New Horizons

ADDRESS OF CONCERN 9110 Red Branch Road, Columbia, Maryland 21045-2014

I hereby declare that the above-identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18 and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled A MEANS FOR THE PROPHYLACTIC AND THERAPEUTIC TREATMENT OF A GROUP A STREPTOCOCCAL INFECTION by inventor(s) Lawrence Loomis and Vincent Fischetti

(X) the specification filed herewith

application serial no. _____, filed _____

() patent no. _____, issued _____

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate: (37 CFR 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: David Trudill

TITLE OF PERSON OTHER THAN OWNER: Vice President

ADDRESS OF PERSON SIGNING: 12616 Mt. Laurel Ct. Reisterstown, MD 21136

SIGNATURE: 

DATE: 10/9/87